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A Study of the Common Cocklebur

Conald P. Abler

A STUDY OF THE COMMON COCKLEBUR

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This is to certify that, in accordance with the requirements of South Dakota State College for the Master of Science Degree, Mr. Conald P. Abler has presented to this committee three bound copies of an acceptable thesis, done in the major field; and has satisfactorily passed a two-hour oral examination on the thesis, the major field, Pharmacy, and the minor field, Zoology.

Advisor

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Head of Major Department

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Rep. of Graduate Committee

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INTRODUCTION

Numerous cases of livestock poisoning due to the common cocklebur have been recorded in the literature during the past three decades. From the stockraiser's standpoint the question of the poisonous properties of this plant has become very important for it grows world wide and is reported to cause heavy loss s annually (1).

Published statements concerning the toxicity of this prolific weed and the nature of the toxic principle, if one exists, are quite contradictory and controversial. The references which state that there is a toxic principle have designated it as a glucoside, an alkaloid, a saponin, and a hydrocarbon. There are many reports in the literature that the plant is innocuous.

Consideration of the economic importance of the cocklebur to the stockraiser and the controversy that exists concerning its toxicity has prompted this study to attempt isolation of a toxic principle from the bur and seed.

HISTORICAL

Reports that cockleburs (Xanthium) are toxic have been made as early as 1881. In his report on "The Chemistry of the Seeds of Xanthium Strumarium", Zander (2) writes of four humans having been poisoned by eating the seeds. In his comprehensive study he found only minute traces of an alkaloid and isolated the glucoside xanthostrumarin. Feeding experiments with the glucoside on cats and frogs showed no toxic symptons. One of his associates, however, became sick after eating some of the seeds.

Lewin (3), 1897, states that Xanthium spinosum in certain stages is poisonous and may kill fifty percent of a herd of hogs or cattle. O'Gara (4) says that one farmer lost twenty pigs which weighed about 160 pounds each. Evidence showed that they had eaten quite a number of young juicy Xanthium burs. "The whole plant as well as the burs is known to contain a poisonous principle which reduces heart action and causes death".

Craig and Bitting (5) stripped young Xanthium plants of the burs and fed them to calves, pigs, rabbits and guinea pigs but no toxic effects were noted. They state that post-mortems of suspected cocklebur poisonings showed irritations of the stomach from the prickly burs and the inflammation resulted in death. Two serious losses of pigs from Xanthium were reported by Mayo (6). Out of 35 head, 25 died in one night and eight more during the following day.

In an article in the "Breeder's Gazette", 1908, Clint (7) says that cocklebur is sure death to hogs. "Farmers sometimes lose 40 percent of their herds."

In 1920 Rhodes, (8), experimentally determining the commercial value of cocklebur oil, found the seed meal or press cake highly toxic. A small quantity of it fed to guinea pigs killed them in one day.

Hansen (9) states that although fatal results are generally attributed to poisoning, there is little evidence to substantiate this belief. He adds that the harmful effects are largely due to the mechanical action of the spiny burs. They may injure by irritating the stomach walls, by lodging in the throat thereby choking the animal, or by clogging the intestinal tract. He believes that overeating of the young and succulent plants may cause death by bloating, which is similar in nature to bloating caused by succulent clover, corn, etc. In a later report by the same author, experiments show the sprouting cotyledons to be toxic. Xanthium burs collected at the site of a hog poisoning were seeded in a selected isolated plot. Before the plant developed true leaves, two young shotes were turned in and permitted to graze at will. They ate ravenously and died in less than 24 hours. These plants, when permitted to develop to the four leaf stage failed to be toxic.

Cattle losses, although less in number than hog poisonings, are also reported. In one herd of 150 cattle, 25 died of Xanthium poisoning; while in another of 58, 20 were reported to have died (10). In the "San Antonio Express" of April 1922, a loss of 15 registered Hereford bulls out of 17 was reported (10).

Marsh, Roe, and Clawson (1) ran numerous feeding experiments with pigs, sheep, cattle and chickens. They fed the whole plants, the young cotyledons, the dry mature fruit, the roots, seeds from dry mature burs, dried cotyledons, stems and roots, seeds from green burs and leaves with cotyledons. The young cotyledons and the dry mature seeds proved to be toxic and fatal. They state that the seeds removed from the burs were much more toxic than any other part of the plant. Their report includes a discussion of symptoms, autopsy findings, microscopic tissue studies, and toxic and lethal dosage determinations, all of which will be discussed later. They found little evidence of mechanical injury due to the whole dried burs. No attempt was made to isolate the poisonous principle. They conclude that the dry mature seeds and the young cotyledons are very toxic.

Seddon and King (11) report that the plant is poisonous to pigs, sheep and cattle, but only in the very young stages of growth and that the toxic principle appears to be concentrated in the cotyledons.

According to Sado (12) aqueous extracts of the stems and leaves of Xanthium contain a substance weakly toxic to nerves and muscles.

Rostelli and Gibelli (13) say that the juice of Xanthium spinosum contains a notable quantity of formic acid which imparts antiseptic properties to it. Formic acid is also present in the dried fruit. "An alkaloid which has an intense pharmacological action on the central nervous system is also found in the dried fruit and in the juice of the fruit bearing plants. As yet the alkaloid has not been isolated in crystalline form".

In the work of Comperi (14) it is reported that the toxic principle appears to be neurohemal in action and is present only in cotyledons of the germinating seed which pigs often obtain by rooting. Its ingestion results in death in a large percent of cases.

Extensive work was done by Krantz, Carr, and Bell (15) in 1943. They prepared a Xanthium extract as follows:

1. Air dry and coarsely comminute the burs.
2. Extract the powder repeatedly with boiling water.
3. Concentrate aqueous percolate at reduced pressure to consistency of a dry mass.

The extract is a light brown color and possesses an odor resembling that of scorched chocolate. It is stable in the air and redissolves partially in water. The yield is approximately 10 percent.

In their feeding studies, four Macacus rhesus monkeys were fed .5 Gm. of Xanthium extract daily over a period of three months.

Blood analysis showed no attributable changes and kidney and liver tissues suffered no significant pathological changes. They add that over a period of two years several hundred thousand .15 Gm. Xanthium extract tablets taken in clinical studies showed no untoward effects.

In determination of pharmacodynamic activity, two percent solutions of Xanthium extracts in normal saline solution were administered intravenously to dogs under nembutal or ether anesthesia. The response elicited was a prompt fall in blood pressure with a rapid return to normal. Animals acquired no tolerance to repeated injections. The fall in blood pressure was not accompanied by any marked change in respiratory rate or volume. Bilateral vagotomy or nicotinization until vagal response was obliterated did not inhibit the characteristic depressor response of the Xanthium solution. The spinal dog likewise showed a fall in blood pressure. Previous injection of atropine obliterated the depressor response. Choline was isolated and identified as the substance responsible for the depressor action.

Chopora and others (16) found that the soluble glucoside from Xanthium is relatively inactive physiologically.

Compori (17) states that a saponin is the toxic principle of Xanthium cavallinessi. He found that quantity of 20 - 30 Gm. of seeds or cotyledons was fatal to rabbits and 10 Gm. of seeds or cotyledons fatal to guinea pigs. Young suckling pigs were killed with five Gm. of seeds or 16 - 31 Gm. of cotyledons.

Carr (18) extracted a fixed oil from Xanthium. Feeding experiments and administration intravenously of the oil and its saponification products gave no evidence of toxicity. He concludes that that portion of the plant is relatively inert pharmacodynamically.

Kuzel and Miller (19) found hydroquinone responsible for the toxic effects produced by injection of aqueous solutions of various species of Xanthium seeds. A 10 percent aqueous extract of the kernels was prepared and found toxic to mice. This extract reduced Benedict's and Fehling's solution. They extracted the seeds with furan and tetrahydrofuran according to the method of Bay and Gisvold. A crystalline substance was obtained. Mixed melting points and carbon and hydrogen determinations indicated the substance was hydroquinone.

BOTANICAL DESCRIPTION AND DISTRIBUTION OF THE COCKLEBUR PLANT.

The cocklebur is an annual of the family Compositae (Daisy family) which produces a stout, rank growth. It is usually one to four feet in height, although the height varies with conditions of the growing season. Specimens of a few inches in height may frequently bear mature fruit, especially late in the fall. Common synonyms are: clotbur, burweed, bathurst bur, sheepbur, bur thistle, button bur, hedgehog bur, and ditch-bur.

Muenscher (20) gives the following description of Xanthium; Annual, stems stout, with spreading branches, rough-pubescent, angled and frequently red spotted. Leaves alternate, simple, long petioled, broadly ovatecordate, dentate or somewhat lobed, rough-pubescent. Heads unisexual, the staminate small in short terminal spikes, several-flowered; pistillate involucre ovoid, corriaceous, closed, hairy, and spiny, bearing two pistillate flowers developing into a hard prickly bur. The bur is oblong, about two centimeters long with numerous hooked prickles and two stout terminal beaks. There are two achenes in each bur; oblong, flattened and dark brown.

The larger of the two enclosed seeds will germinate the first year. The smaller usually requires two or more seasons before it will germinate. This fact has led many to assume the plant to be biennial or perennial in nature. Several very similar forms, based largely on variations in the shape, hairiness, and spines of the mature burs, are frequently designated as separate species. Marsh (1) states that

there is little doubt that in the literature on the subject there has been much confusion in the use of specific names and the designation of specific limitations.

The species most often referred to in the literature are Xanthium spinosum, Xanthium canadense, anthium orientale, Xanthium echinatum, Xanthium commune, Xanthium strumarium, and Xanthium chinense. The species of Xanthium are widely distributed throughout the world. They grow profusely in moist waste places, barnyards, pastures and along the shores of rivers, lakes, ditches, and ponds. They are exceedingly abundant in marshy lands and often cover hundreds of acres (21). The fact that these plants grow along water shorelines is of particular importance to the stockraiser. Throughout the entire growing season, as these waters recede, there is at all times a growth of young succulent plants present upon which the livestock may feed.

TOXICOLOGY

Zander (2) quotes from a letter received from Heimberger in 1880 which told of human fatalities occurring after eating the seeds of Xanthium. Four children became violently ill after consuming an unknown amount of the seeds. Three of the cases resulted in death within twenty four hours. The fourth recovered after a serious illness. The symptoms noted were initial depression with a weak, rapid pulse and pronounced dyspnea, followed by extreme nausea and noticeable body tremors. Post-mortem examination showed froth in the esophagus, pericardium infiltrated with excessive serous fluids, enlarged spleen, engorged kidneys and hardened liver. In one instance the heart was in ventricular systole.

In a typical case of acute poisoning of pigs the initial depression is accompanied by nausea which frequently results in continued vomiting. The animal becomes so weak it is unable to stand. Respiration is labored and there is difficulty in breathing. The pulse is rapid and weak and there are occasionally spasmodic movements of the body. There is depression of body temperature. Often the animal moves its legs in what may be described as running movements. Poisoned cattle and sheep react much the same as pigs, but there is no vomiting. Occasional hyperesthesia is also noted. "The symptoms ordinarilly appear within twenty four hours after the plant is eaten and commonly continue for only a few hours" (1).

Kusel and Miller (18) injected mice intraperitoneally with an aqueous extract of Xanthium seeds and with hydroquinone isolated from this extract. The animals showed a marked irritation at the site of injection accompanied by increased diuresis. There were body tremors which turned into coordinated hopping movements, culminating in frenzied running about the cage. The animal became ataxic and lay on its side still continuing its running movements. Just before death the animal became spasmodic, relaxing after death. The pig showed initial depression accompanied by bloody foaming at the mouth, repeated urination, body tremors, dyspnea, and the characteristic running movements followed by death. They state that oral administration generally increased the fatality.

Autopsy findings reveal numerous muscular and subcutaneous hemorrhages. The lungs are often collapsed and may be off color. The liver is congested with local hemorrhages, the kidneys and spleen may be engorged with blood and fluids. The intestines are red and congested and there is great serous infiltration of most body cavities. Various abnormalities of the heart may be observed.

Microscopic tissue studies by Marsh (1) showed hemorrhages in all liver lobules and a severe necrosis of the hepatic cells. Gall bladder walls were sometimes necrotic. In the kidneys a parenchymatous nephritis affected the convoluted tubules and the ascending loop of Henle. Sections of the stomach wall showed a pronounced capillary congestion and hemorrhagic condition of the mucous membrane. The intestines

showed no uniform pathologic change. Spleen sections showed signs of irritation and congestion. Heart and lung tissues showed no changes of significance. "Lung specimens were characterized by a lack of blood rather than congestion. This was probably due to the removal of blood from the general circulation which had accumulated in the liver" (1).

Marsh (1) gives an approximate toxic and lethal dose for the cotyledons and the seeds. In experiments on pigs the smallest toxic dose of cotyledons was .736 percent of the weight of the animal. The smallest lethal dose was 1.496 percent of body weight. "Thus the toxic dose of cotyledons to pigs is about one half that of the lethal dose".

The dry mature seeds, in one instance, killed a pig when eaten in an amount equivalent to .275 percent of body weight, while another pig consumed .25 percent of its body weight without effect. Marsh then states that it is probable that the toxic and the lethal dose of the mature seed is not far from .275 percent. "Experiments seem to indicate that there may be an acquired tolerance to continued feeding of the cotyledons". He adds, however, that their experiments were not sufficient enough in number that a positive statement could be made to this effect but that it seemed probable.

Administration of fats and oils seems to have a beneficial effect in cases of Xanthium poisoning. It is believed that they act by preventing or delaying absorption of the toxic substance. Marsh (1)

administered one and one half quarts of milk to three pigs that had consumed an amount of young cotyledons in excess of 1.5 percent of animal weight. The animals suffered no symptoms. Sixty Gm. of lard and 60 cc of raw linseed oil also prevented poisoning of pigs receiving a lethal dose of cotyledons.

PROPERTIES OF XANTHIUM BURS AND SEEDS

Kuzel and Miller (19) made a comparative chemical assay of Xanthium burs and seeds. They found the burs to contain 5.45 percent total ash, 2.2 percent total nitrogen, 13.75 percent crude protein, 17.08 percent crude fat, and 53.59 percent crude fiber. The kernels contained 6.05 total ash, 6.83 percent total nitrogen, 42.76 percent crude protein, 32.8 percent crude fat and 2.75 percent crude fiber.

Xanthium fruit, particularly the seed, contains a considerable amount of fixed oil resembling that of sunflower oil. Tussing and Dunbar (22) found that the whole bur contained 4.6 to 7.5 percent of this oil by weight. Branke and Gutt (23) found the seeds to contain about 40 percent fixed oil by weight, while Chopora, Kohli, and Handa (16) say the seeds contain 32 percent of the oil by weight. Rhodes (8) found Xanthium oil a good drying oil. Xanthium oil completely dried in five days, while pure raw linseed oil dried in four days.

The oil has a saponification value of 189-205, an iodine value of 136-137, a Reichert-Meissel value of 1.04-1.07, a Polenski value of .16-.18, and contains a .85-.91 percent unsaponifiable matter. Its acid value ranges from .43-.66 and it has a density of .893-.919. Xanthium oil contains 26.7-27.1 percent oleic acid, 63.4-64.8 percent linoleic acid, and small amounts of stearic and palmitic acids (22, 23, 24).

Hansen (9) says that cocklebur oil has been used in paints and varnishes and in some cases as a human food. The cake resulting from pressure extraction of the oil is utilized for feed and fertilizer in the same manner as cottonseed cake.

EXPERIMENTAL

Materials Used

The Xanthium burs used in these experiments were obtained from two different localities in South Dakota. The largest quantity, about five bushels by volume, was collected from two different fields on a farm located three miles southwest of Brookings. One field was an uncultivated hog yard, the other a harvested millet field. The plants in the hog yard ranged from two to four feet in height, while those in the millet field averaged about 12 inches in height. In both areas the plants had developed mature fruit, although the smaller plants yielded fewer large burs.

A second quantity of burs, two bushels in volume, was collected from a farm twelve miles southwest of Seneca, S. Dak. in southwestern Faulk County. These burs were obtained from plants found in ditches, ravines and waste, uncultivated land. The plants ranged from one to four feet in height.

All collections were made after several killing frosts had occurred and the plants had begun to wither and die. Most burs had turned a dark brown or black color and only a small percentage of them retained a green color. The plants were uprooted and stripped of all the burs. No attempt was made to grade the burs in any way at the time of collection. It was found that a bushel measure packed tightly with burs averaged 10 pounds in weight.

Collection Table

Date	Amount	Locality.
10-2-49	24 pounds	Brookings, S. Dak.
10-7-49	6 pounds	"
10-8-49	8 pounds	"
10-14-49	8 pounds	"
10-15-49 to 10-25-49	15 pounds	Seneca, S. Dak.

The material submitted to the Department of Botany, S. D. State College, for identification corresponds well with Xanthium italicum, Moretti, as described in Gray's Manual of Botany, 8th edition (25). It should be noted, however, that Wiegand (26) was inclined to combine this and several other allied forms under the specific name Xanthium orientale, Linne. Present state of taxonomic research will not permit a final definitive decision as to how comprehensive Xanthium orientale L. really is.

The crude drug was air dried at room temperature until needed. A period of at least three months drying was necessary before the drug was sufficiently dry to grind.

Normally each bur will contain two seeds, one a bit smaller than the other. Upon examination it was found that some of the burs collected contained only one seed and others were empty. Rhodes (8) states that in removing the seeds from selected burs he was able to obtain a yield of approximately 30 percent by weight. In experiment it was found that seeds obtained from the burs collected for this project accounted for about 15 percent of the total bur weight. No selection was made and burs used were just as obtained from the field.

Weight Table

Sample	Weight of Burs	Weight of Seeds Obtained	Percent Yield
1	300 Grams	44.6 Grams	14.87
2	300 "	45.8 "	15.27
3	200 "	30.4 "	15.20

Preliminary Examinations and Tests

Since the literature in general indicates that the toxic principle is water soluble we decided that preliminary tests should be made on aqueous portions of the drug.

A 10 Gm. sample of the crude drug was warmed with water in a water bath to 60° C. for 20 minutes, then cooled and filtered. The extract was a cloudy dark brown liquid, very difficult to clarify by ordinary filtration. It was acidic in reaction to litmus paper. Ferric chloride T.S. added to the extract caused formation of a dark brown gelatinous precipitate which settled slowly leaving the supernatant liquid clear and colorless.

Lead Acetate T.S. added to the aqueous extract gave a brownish precipitate very similar to that formed with ferric chloride. It also settled out slowly and the supernatant liquid was clear and without color. The precipitate was removed by filtration and discarded and the resulting filtrate treated with Lead Subacetate T.S.. A slight turbidity appeared but no definite precipitate formed.

Heating the extract with Benedict's Solution and Fehling's Solution for 30 minutes showed only minute traces of reduction. The same tests were positive after the extract was hydrolyzed with HCL for 20 minutes in a boiling water bath.

A small portion of the crude drug shaken vigorously with sodium carbonate ten minutes produced a slight froth which persisted for one hour.

The aqueous extract after hydrolysis with HCL was treated with phenylhydrazine hydrocholoride and sodium acetate. Osazone crystals were formed. Examination under a microscope showed the typical sheaf like crystal formations of glucosazone. The lead acetate filtrate described above produced the same osazone crystals after hydrolysis with HCL. Neither the aqueous extract nor the lead acetate filtrate would yield osazone crystals without previous hydrolysis with HCL.

A preliminary test was made for the presence of an alkaloid. A portion of the comminuted Xanthium bur was extracted with a one percent cold hydrochloric acid solution and filtered which yielded an extract light brown in color. It was then made alkaline with Ammonia T. S.. Upon addition of the ammonia the extract turned a dark brown color. It was then extracted with ether and the ethereal solution evaporated to dryness on a water bath. The slight residue was taken up with acidulated water. Mayer's Reagent, Phosphotungstic Acid Solution, Wagner's Reagent, and Saturated Picric Acid Solution added to portions of the aqueous solution gave negative results.

Extraction Studies.

It was decided to obtain the fixed oil of Xanthium to determine the amount present. The crude drug was macerated for 24 hours and then percolated with petroleum ether until extraction was complete. The percolate was a light yellow color and contained the fixed oil of Xanthium. This oil was obtained by spontaneous evaporation of the percolate. It was found that 500 Gm. of drug yielded 20 Gm. of Xanthium oil or a yield of 4 percent. The oil thus obtained was tested for the presence of an alkaloid. It was redissolved in petroleum ether and shaken out with several quantities of acidulated water. The aqueous layer was drained off and made distinctly alkaline. It was then extracted with chloroform, the chloroform evaporated, and the residue taken up with acidulated water. Mayer's Reagent, Wagner's Reagent, Phosphotungstic Acid Solution, and Saturated Picric Acid Solution added to the aqueous solution gave no precipitation reactions.

An alkaloid extraction of the crude drug was carried out. The method as given by Belladonna Leaf, U.S.P. XIII, was used. Twenty Gm. of crude drug were moistened with a mixture of Stronger Ammonia T.S., alcohol, and ether and permitted to macerate over night. Extraction was carried out to completion with a Soxhlet apparatus using a mixture of three volumes of ether and one volume of chloroform as menstruum. The percolate was extracted with quantities of acidulated water in a separatory funnel. The aqueous solution then alkalinized was extracted with successive portions of chloroform. The chloroform

solution was evaporated to dryness and the residue dissolved in acidulated water. To this solution the following reagents were added:

IKI Solution----------Rust brown precipitate.

Saturated Picric Acid Solution--------Yellow precipitate.

Phosphotungstic Acid Solution---------White turbidity.

Mayer's Reagent----------White precipitate.

The precipitates redissolved on standing with the exception of the IKI precipitate which partially redissolved. A second experiment carried out in the same manner as above gave negative results with the alkaloidal reagents.

Pharmacological Studies

An aqueous extract of Xanthium burs was prepared to be administered intravenously to anesthetized dogs in an effort to observe its effect on blood pressure and respiration. The extract was prepared as follows: The coarsely ground burs were extracted with hot distilled water until the liquid when strained off appeared quite dilute. The liquid was filtered while warm and concentrated at reduced pressure to the consistency of a soft syrup. It was then spread on a pill tile to dry spontaneously. The residue was a dark brown color and dried to the consistency of a plastic mass. It was redissolved in distilled water and clarified by filtration. The yield was about 10 percent.

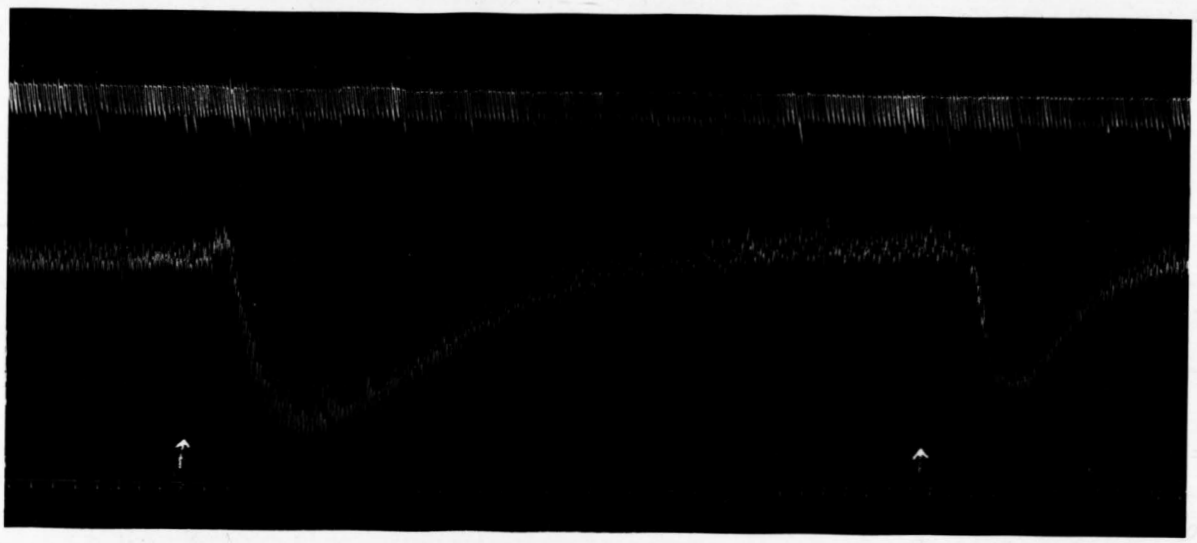
In the experiments two percent solutions were used for all intravenous injections of the Xanthium extract. Injections were made via the left femoral vein with a gravity flow cannula hookup. The right carotid artery was entered for blood pressure readings and respiration tracings, when taken, were made using the thoracic cavity puncture. Nembutal anesthesia was used exclusively on all dogs.

Three milliliters of Xanthium extract administered in Experiment I (Fig. 1) elicited an immediate fall in blood pressure. Blood pressure returned to the normal level rapidly. Administration of 10 mg. of choline chloride prompted a response very similar in magnitude and character. Complete atropinization was then inaugurated by injection of one milligram of atropine sulfate. The atropine was then followed by 10 mg. of choline chloride which showed only the nicotinic effect and no fall in blood

pressure indicating an atropine block. The original quantity of Xanthium extract then administered still brought about a depression of blood pressure. Experiment II (Fig. 2), shown in entirety, shows a similar procedure with corresponding results as did experiments with four other dogs.

One of the Xanthium solutions which was permitted to stand exposed to light and air for ten days was observed to have lightened in color and formed a precipitate. The extract, after filtration, failed to elicit a blood pressure response as before. The residue suspended in physiological saline solution and injected intravenously also failed to evoke a fall in blood pressure.

At this time the work of Kuzel and Miller (19) proclaiming hydroquinone as the toxic factor in Xanthium was published. For this reason it was decided to see if hydroquinone might possibly account for the non-blockable pressure response described above which was not evidenced in the work of Krantz, Carr, and Bell (15). Amounts of one, two, and five milliliters of a two percent hydroquinone solution in saline were injected intravenously. None of the three amounts evoked a blood pressure response. Experiment III (Fig. 3) shows the administration of 5 cc. of a two percent hydroquinone solution compared with the administration of 3 cc. of Xanthium extract.



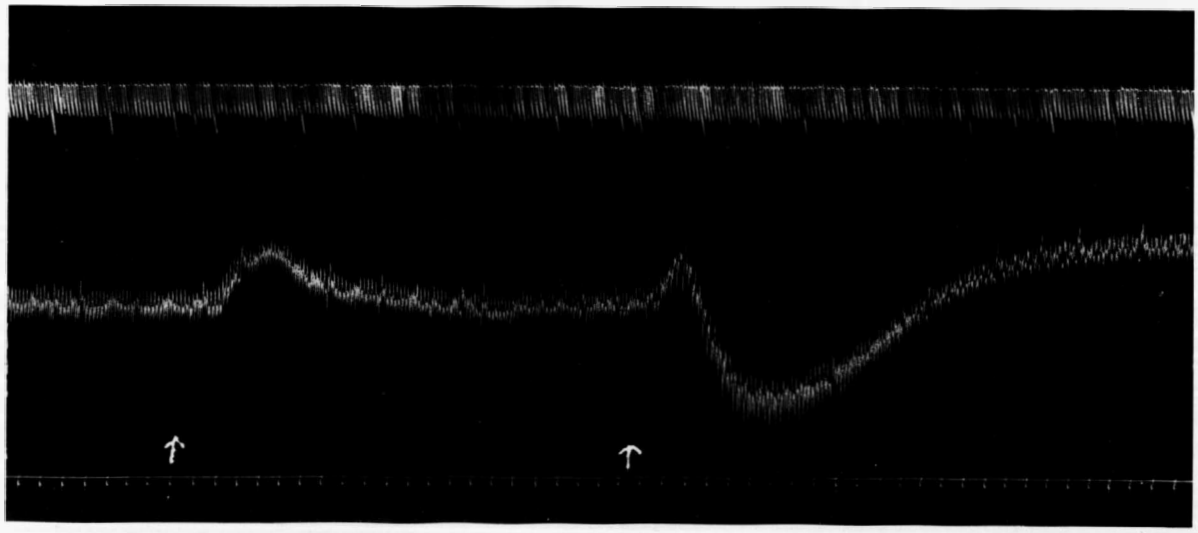


Fig. 1.

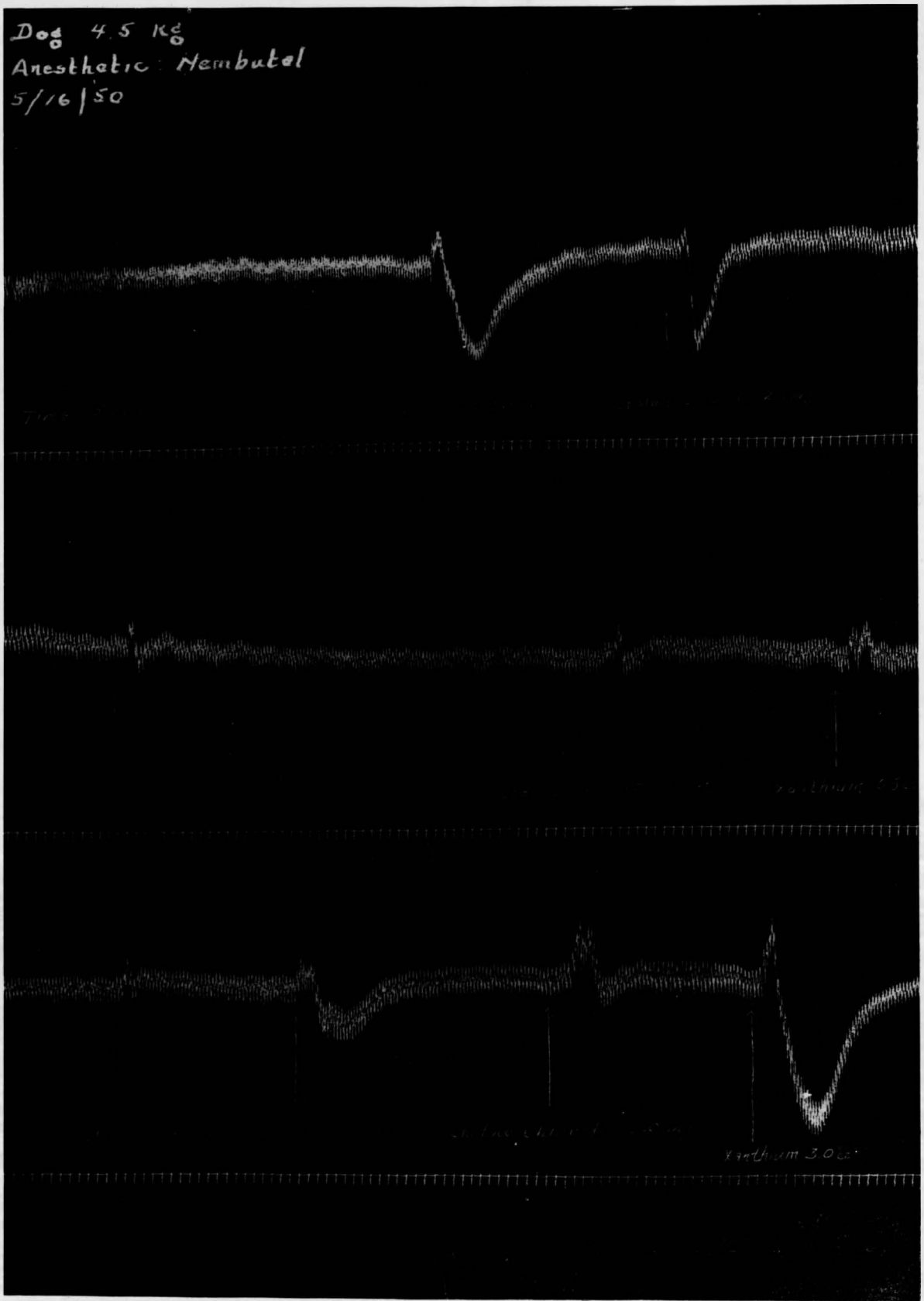
Above—3 cc. of Xanthium Extract and 10 mg. of choline chloride respectively before atropine.

Below—10 mg. of choline chloride and 3 cc. of Xanthium Extract respectively after atropine.

Specimen: Nembutal anesthetized dog.

Time Interval: Five seconds.

The accompanying photograph (Fig. 2.) shows administration of Xanthium Extract and choline chloride before and after atropine.


Dog 4.5 Kg
Anesthetic Nembutal
5/16/50

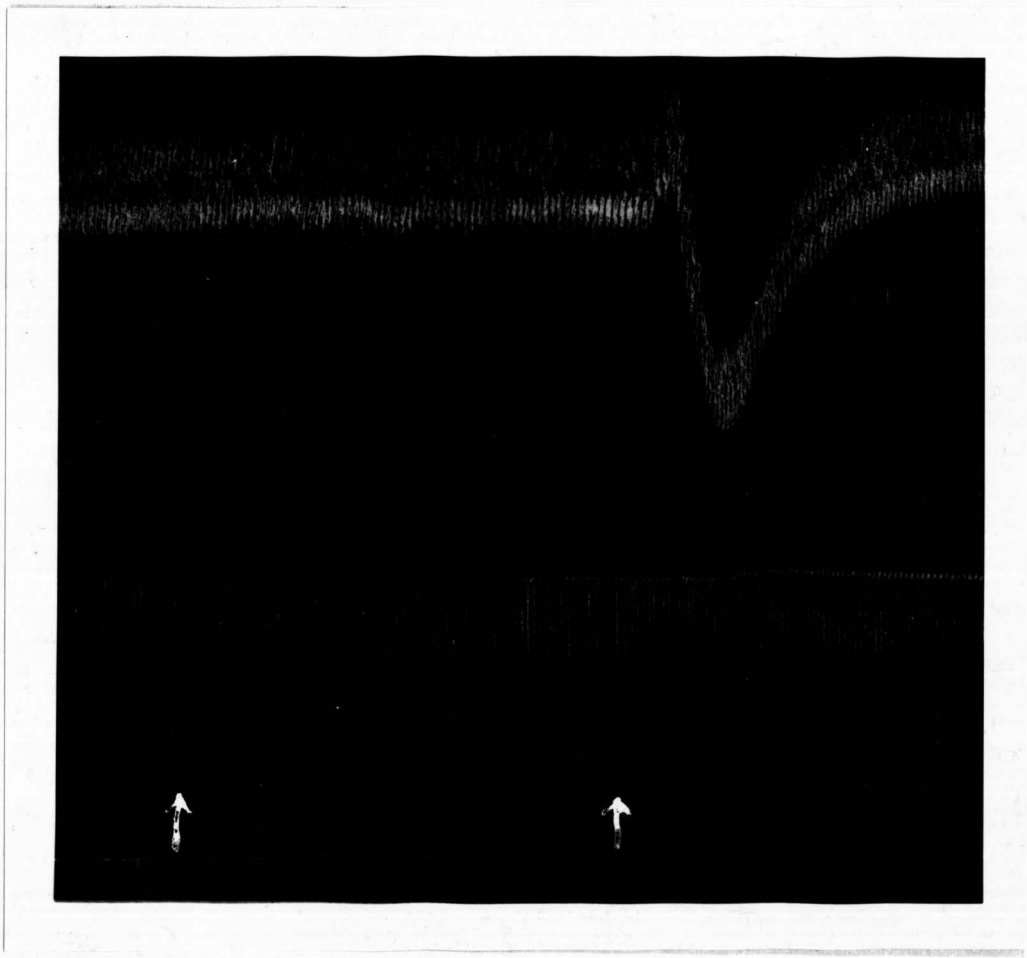


Fig. 3.
Shows administration of 100 mg. of hydroquinone and 3 cc. of Xanthium Extract respectively.
Specimen: Nembutal anesthetized dog.
Time Interval: Five Seconds.

Feeding Experiments.

Feeding experiments involving the entire bur, the seeds, and the young green plant were conducted on rabbits and male white rats in an attempt to determine what quantity of these materials, when ingested orally, would produce toxic symptoms or death.

It was anticipated that it might be difficult to induce animals to consume Xanthium burs since their odor is very unpleasant, particularly when in the ground state. A male rabbit and a male white rat were selected to determine the steps necessary to persuade animals to consume a Xanthium bur diet. Both animals were starved for 48 hours and given portions of the ground bur to eat. The materials was refused. Th n a mixture of four parts of ground Xanthium burs and one part of ground balanced ration was put before them and it was also refused. On the fifth day when the mixture was changed to two parts Xanthium and three parts chow the animals ate hesitatingly. This feed mixture was kept before them continuously for three weeks. In this three week period the rabbit consumed 550 Gm. of the Xanthium mixture and the rat ate 180 Gm. of the mixture. This represents 220 Gm. and 72 Gm. of Xanthium burs respectively. Neither animal showed any untoward symptoms, however, they always appeared very hungry.

In feeding experiment I (Table 1), ten rabbits (seven females and three males) ranging from 1.69 Kg. to 3.19 Kg. in weight were used. The diet was changed to a mixture of equal parts of ground Xanthium burs and

ground balanced ration in an effort to increase the daily consumption of Xanthium. The amount given to each animal was weighed and recorded and the amount remaining in each feeding tray the following day was weighed and daily consumption recorded. The Xanthium mixture was kept before the animals at all times, occasionally necessitating three feedings per day. Special feeding trays, consisting of a smaller and shallower can mounted inside a coffee tin, improvised to prevent waste, proved very efficient. Some of the animals ate sparingly the first day but gradually became accustomed to eating the diet and at the end of the experiment were consuming daily amounts up to four times the daily quantity eaten during the first few days of the experiment.

None of the animals showed any untoward symptoms throughout the experiment except Rabbit 3 which became sick on the tenth day and died on the twelfth day. A doubt exists whether or not this animal died from Xanthium poisoning. All animals lost weight during the experiment, one as little as .05 Kg., another as much as .49 Kg..

Since no toxic symptoms other than a loss in weight were observed in animals ingesting the amounts of Xanthium listed in Table 1, it was decided to administer a concentrated aqueous extract of the bur to smaller animals, thereby greatly increasing the amount consumed in terms of weight of Xanthium bur per kilogram of body weight.

An aqueous extraction of 1000 Gm. of Xanthium burs was concentrated under reduced pressure to 400 cc. in volume. This solution, in which 1 cc. represented 2.5 Gm. of the crude drug, was administered orally to male white rats by means of a small hard rubber catheter. Five adult male

rats each received as high as 10 cc. of this extract daily for eight consecutive days (Table 2). The animals were permitted to eat and drink as usual. No symptoms or fatalities occurred throughout the experiment.

Since the seed is often referred to as the most toxic portion of Xanthium (1, 8, 13, 14, 17, 19, 20), and since experiment had shown us that the seeds comprised only 15 percent of the total bur weight, it was possible to increase ingestion of a probable toxic principle still more in terms of Gm./Kg. of body weight by feeding the seeds of Xanthium. In Experiment III, (Table 3), adult male white rats ranging from 265 Gm. to 305 Gm. in weight were fed a diet containing Xanthium seeds for eight days, daily consumption being calculated for each animal as in Table 1. The composition of the diet used was two parts of finely ground Xanthium seeds mixed with one part of finely ground lab chow. All animals gained weight during the experiment. Gains from 1 to 30 Gm. per individual were recorded. No symptoms of poisoning were observed in any of the animals.

Because no toxicity was demonstrated in the feeding experiments thus far, it seemed advisable to find out if the young plants may be toxic. These were grown from seeds removed from the same lots of burs used in the other feeding experiments. According to Kinch (28) his experimental work had shown that 35 to 40 percent germination of greenhouse planted seeds was the usual obtained. He recommended extended chilling of the seeds after removal from the burs and germination in a cool dark place, transferring to a greenhouse after sprouting began. It was found that about the fourth day after planting the plants began to break

through the soil. In the plantings conducted 58 and 75 percent germinations were encountered. The seeds used in the second planting were refrigerated for 14 days while those of the first only three days.

On the tenth day the plants were all uprooted and fed, thus some germinating cotyledons were included with the young plants in the feeding experiment. None of the plants had yet reached the stage of having true leaves. The whole plants were fed to rabbits and consumed readily at one feeding. None of the animals showed any symptoms of poisoning.

Rabbit No.	Sex	Animal Wt. in Kg.	Grams of Xanthium Mixture* Fed and Consumed Daily** 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Gram Total Consumed	Animal Wt. in Kg.	Kg. Loss in Weight
1	F	1.78	30/0	30/9	30/9	30/12	65/31	45/21	45/19	60/23	45/28	60/34	60/33	60/41	60/38	90/58	356	1.52	.26
2	F	2.13	30/0	30/20	30/11	30/18	65/30	45/34	45/29	60/39	45/43	60/57	60/27	75/70	60/57	90/78	513	1.80	.33
3	F	1.69	30/21	30/6	30/8	30/20	65/33	45/26	45/20	60/19	45/25	60/10	60/0		Died		188	1.14	.55
4	F	2.60	30/20	45/22	30/22	45/42	69/66	90/50	75/32	60/41	60/54	90/55	75/37	75/56	90/40	90/52	589	2.11	.49
5	M	3.17	30/15	30/20	30/18	30/20	57/46	45/31	45/29	75/51	45/33	60/42	60/41	90/70	60/42	90/83	541	2.86	.31
6	M	2.87	30/14	45/16	30/18	30/19	95/56	75/64	60/25	90/55	60/29	60/36	60/37	80/64	60/37	90/77	547	2.52	.35
7	M	2.96	30/23	30/22	45/25	30/25	65/50	75/62	45/40	75/65	45/42	60/54	60/57	90/86	60/56	90/85	692	2.91	.05
8	F	2.00	30/22	30/22	30/22	30/29	65/62	45/42	75/42	75/68	60/39	60/52	60/53	90/85	60/54	86/81	673	1.96	.04
9	F	2.33	30/23	30/22	30/10	30/11	65/8	56/27	45/13	60/16	45/18	47/19	60/27	60/24	60/22	75/58	298	1.95	.38
10	F	2.31	30/22	30/21	30/19	30/26	65/49	45/25	45/38	75/52	60/48	75/50	60/51	60/36	60/55	75/64	556	2.21	.10

Feeding Table No. 4.

*Mixture is one part ground Xanthium burs and one part ground balanced rabbit ration.
**Example 30/20 indicates 30 Gms. fed 20 Gms. consumed on given day.

Rat No.	Gram Weight Before	cc. of Xanthium Extract Fed Daily								Total in cc.	Gram Weight After
		1	2	3	4	5	6	7	8		
1	285	2	6	7	8	10	10	10	10	63	287
2	278	4	6	7	8	10	10	10	10	65	277
3	302	5	6	7	8	10	10	10	10	66	302
4	267	5	6	7	8	10	10	10	10	66	267
5	276	6	6	7	8	10	10	10	10	66	274

Feeding Table No. 2

Rat No.	Gram Weight Before	Grams of Xanthium Seed Mixture* Consumed Daily							Total g	Weight Eaten	Weight After
		1	2	3	4	5	6	7			
6	305	15	15	14	15	16	16	17	16	124	325
7	266	0	0	2	2	10	15	15	13	57	266
8	285	15	12	14	15	17	16	14	16	119	285
9	280	0	0	0	10	11	14	18	21	74	280
10	265	12	14	15	15	14	18	20	16	124	265

*Mixture is two parts ground Xanthium seed and one part lab chow.

Feeding Table No. 3.

Rabbit No.	Kg. Weight	No. Plants Eaten	Plant Weight in Gms.
11	3.12	24	13
12	2.55	76	40
13	2.25	106	63.8

Feeding Table No. 4.

Studies of Xanthium Seeds

In various instances in the literature it is reported that the seeds of Xanthium are or may be more toxic than other portions of the plant. In view of this information and in consideration of the fact that the whole bur presented more difficulties in extraction, it seemed advisable to confine the search for a toxic principle to the seeds removed from the burs.

A search of the seeds for an alkaloid according to the general methods of U. S. P. XIII gave negative results as it did with the studies of the bur.

Although we were not able to demonstrate toxicity by feeding Xanthium in various forms, and since Kuzel and Miller (19) found hydroquinone present in the Xanthium that they studied, it seemed advisable to find out if hydroquinone was at all present in the Xanthium which we were using. The extraction method of Bay and Gisvold (27) was used.

The procedure was as follows: Twenty five grams of Xanthium seeds and 250 cc. of cold distilled water were placed in a Waring Blendor and mixed at high speed for ten minutes. The mixture was warmed to 60 C. and filtered. The filtrate was concentrated at reduced pressure to about 20 cc., saturated with sodium sulfate, and 2 Gm. of charcoal added. The mixture was dried over sulfuric acid. The residue was then extracted with tetrahydrofuran in a Soxhlet apparatus for 16 - 24 hours. The percolate was evaporated on a water

bath, avoiding inhalation of the toxic fumes of the solvent. Substitution of tetrahydrofuran with ether and alcohol in other extractions did not prove satisfactory.

The brownish, greasy, crystalline deposit was taken up with ether and filtered. The ether solution upon evaporation left shining yellow crystals. The crystals were washed with chloroform several times to remove the yellow color and then allowed to crystallize spontaneously from alcohol. Long, shiny, white, needlelike crystals were obtained. Their crystalline structure when observed under a microscope compared favorably with that of hydroquinone.

The quantity of these crystals obtained was very small. A 50 Gm. sample of the seeds yielded 100 mg. of crystals while a second sample of 75 Gm. yielded 122 mg. of crystals. This was a yield of .2 percent and .16 percent respectively.

The crystalline material was soluble in alcohol, ether, petroleum ether, and water, and was insoluble in chloroform. It reacted readily with nitric acid with the evolution of the oxides of nitrogen. Concentrated sulfuric acid added slowly to an aqueous solution of the crystals produced a greenish brown ring at the junction of the two layers. An aqueous solution reduced Fehling's and Benedict's solutions but attempts to form an osazone failed. Aqueous solutions turned brown on continued exposure to light and air. This color reaction occurred immediately upon the addition of sodium carbonate and cleared up again on the addition of an excess of acid. Addition of ferric

chloride T. S. failed to give a color reaction. The solubility properties and chemical tests stated above were identical to those run on a sample of pure hydroquinone.

A melting point determination showed that the substance melted at 168° - 169° C. as compared to 169° C. for hydroquinone. A diacetate derivative of the unknown was successfully prepared according to the method of Shriner and Fuson (29). It melted at 121° - 122°C. The diacetate derivative of a known hydroquinone sample melted at 122° C. as compared with 123° C. quoted in the literature.

SUMMARY

1. About .2 percent of hydroquinone was found in Xanthium italicum, Moretti. The diacetate derivative was successfully prepared.

2. There was no evidence of an alkaloid.

3. An osazone was formed from the aqueous extract of the burs after hydrolysis with hydrochloric acid.

4. A depressor response similar to that of choline was effected by intravenous injection of an aqueous Xanthium extract. Atropine would not block this response.

5. Feeding experiments conducted on rats and rabbits failed to show toxicity in the amounts eaten. Over a two week period rabbits consumed ground Xanthium burs in amounts ranging from 10 - 25 Gms. per day per animal or in doses of 4.3 - 8.4 Gms. per Kilogram body weight. Male white rats were force fed an aqueous extract of Xanthium burs (1 cc. equivalent to 2.5 Gms.). This represented average daily doses of 20.5 Gms. of Xanthium burs or 72.9 Gm./Kg.. Xanthium seeds were eaten by rats in amounts of 10.25 Gms. daily, representing about 38.7 Gm./Kg.. A 2.25 Kg. rabbit ate 63.8 Gms. of young Xanthium plants without effect.

CONCLUSION

Experimental work has shown hydroquinone to be present in small quantity in lots of Xanthium italicum, Moretti, collected from two localities in South Dakota. No evidence of an alkaloid could be found. An osazone, probably glucosazone, was formed from an aqueous Xanthium extract only after hydrolysis with HCl. It was found that an aqueous extract of the bur and seed contained a depressor principle similar to choline in action. However, it was not possible to block this depressor action with atropine. This action is not due to the hydroquinone present.

Feeding experiments of the bur, seed, and young plants of Xanthium on rats and rabbits failed to show toxic symptoms other than a loss of weight. It is apparent that the quantity of hydroquinone found in these lots of Xanthium was not sufficient to poison rats or rabbits in the amounts consumed.

Additional research is necessary to determine if factors such as the locality, species differences, or conditions under which Xanthium is grown may be responsible for the contradictory statements concerning the toxicity of the cocklebur.

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